RESEARCH PROPOSAL FOR TOPIC APPROVAL TO BE SUBMITTED TO THE UNIVERSITY OF MUMBAI FOR THE DOCTOR OF PHILOSOPHY (SCIENCE) DEGREE IN THE SUBJECT OF ~~BIOTECHNOLOGY~~ BOTANY

Title of the topic	:	**Studies in *Mammea suriga* (Buch. -Ham. ex Roxb.) Kosterm with special reference to morphological variability and ability of various plant parts to inhibit malodor and acne causing microorganisms.**
Name of the student	:	Mrs. Mamta Subodh Nagwekar (Ms. Mamta Patil)
Basic Qualification	:	M.Sc. Biotechnology
In case of Ph.D. student Year of Passing PET	:	2020
Name of the Research Guide	:	Prof. Dr. Ajit Kengar
Name of the Research Centre	:	Botany Research Lab, Department of Botany, KET's V. G. College of Arts, Science and Commerce (Autonomous) Mithagar Road, Mulund (E), Mumbai 400081
Date of Superannuation/ Retirement of the Research Guide		30th June 2032
Signature of the Student	:	M.Nagwekar
Signature of the Research Guide	:	

Date: 25th April 2023

Seal of College/Department

K.E.T.'S VAZE COLLEGE 1984

Signature of

Head/Director/Principal Research Centre

Principal
V. G. Vaze College (Autonomous)
Mulund (East), Mumbai - 400 081.

INDEX

Sr. No.	Content	Page no.
1	Title	
2	Aims	
3	Objectives	
4	Background (Literature Review) and Significance of study	
5	National Status	
6	International Status	
7	Hypothesis	
8	Rationale	
9	Research Methodology	
10	Statistical Plan	
11	Limitations of the Study	
12	Expected Outcomes	
13	Select Bibliography	

TITLE

Studies in *Mammea suriga* (Buch. -Ham. ex Roxb.) Kosterm with special reference to morphological variability and ability of various plant parts to inhibit malodor/acne causing microorganisms.

AIMS

A) Biodiversity studies in *M. suriga* including morphological variations.

B) Extraction of Bioactives from various parts of *M. Suriga,* Fractionation with Column chromatography and evaluation of various fractions for inhibition of malodor/acne causing microorganisms.

OBJECTIVES

1. Biodiversity study of *M. Suriga* including morphological characteristics like Tree height, Tree stem girth, Leaf morphology, Floral structure and Pollen structure.
2. Extraction of Concrete Fragrant Molecule from dry and fresh flowers of *M. Suriga* obtained from 2 geographical locations of Kokan by using different solvents will be subjected for Olfactory evaluation and GC-MS analysis.
3. Extraction of active components from flowers, buds, leaves and seeds of *M. suriga* using various solvents
4. Evaluation of antimicrobial activity of the extracts against malodour causing micro-organisms (*Staphylococcus epidermidis* and *Corynebacterium xerosis*) and anti-acne activity against *Propionibacterium acne*.
5. Fractionation of shortlisted extracts with Column chromatography and evaluation of various fractions for inhibition of malodor/acne causing microorganisms. Characterization of active component responsible for antimicrobial activity
6. Evaluation of *in-vitro* cytotoxicity of extracts on HDF (Human Dermal Fibroblast) cell line.
7. Evaluation of anti-proliferative activity of the extracts on cancer cell lines.
8. Antioxidant capacity of *M. Suriga* extracts by DPPH method.
9. Extraction of polysaccharides from seeds of *M. suriga*.
10. Screening Moisturising ability of these polysaccharides
11. Incorporation of extracts in suitable cosmetic formulations

BACKGROUND AND SIGNIFICANCE

Need for Biodiversity study

India is a country of multi diversity having high Himalayas to sea level area and world highest rainy area to Thar Desert (Keshari, 2022). Due to vast changes in temperature and precipitation, India has a wide range of climatic conditions (tropical, subtropical, temperate, alpine, and so on). Climate change has resulted in an abundance of flora and fauna, making the country a "mega biodiversity country" in the globe. Four of the 35 global hotspots discovered to date are in India: The Western Ghats, Himalaya, Indo-Burma, and Sundaland. (Tripathi, 2015) Western ghat is a UNESCO World Heritage Site and the area is one of the world's ten "hottest biodiversity hotspots." It has over 7,402 species of flowering plants, 1,814 species of non-flowering plants It is likely that many undiscovered species live in the Western Ghats. At least 325 globally threatened species occur in the Western Ghats.

M. Suriga is one of the narrowly endemic, critically endangered plant species of western ghats with an extremely restricted distribution, limited to small populations found in only districts like Sawantwadi, Vengurle and Malvan. Species that have small population sizes and a restricted geographic range (i.e., narrow endemic) tend to show lower levels of diversity than those with larger population sizes and wide distribution (Kimura and Crow, 1964; Hamrick and Godt, 1989; Honnay and Jacquemyn, 2007). Low levels of genetic diversity has been reported for threatened plant species with a history of fragmentation and/or population decline (Jiménez et al., 2014; Bupp et al., 2017; Simmons et al., 2018; Downey and Baskauf, 2020). In addition, high levels of inbreeding are expected for plant species with small and isolated populations. As a result, such populations are particularly prone to extinction, as they lose variability more readily. Hence it is very essential to undertake the systematic diversity study among the populations for *M. suriga*. Such study will tackle a central issue in evolution and conservation biology and we expect that it will be useful to safeguard the genetic diversity reported for the only remaining population of *M. suriga*. The approach applied in this study can also be used to guide conservation efforts for other plant species on the brink of extinction.

According to the Bulletin of the World Health Organization (WHO), around 65% of the world's population relied on medicinal plants as their primary healthcare Source. Also, it has been observed that about 50% of the medicines developed since 1980 have been natural products, their derivatives, or their analogs (Varpoorte 2010). Amongst them, analgesics (morphine),

cardiotonics (digoxin), anticancer drugs (paclitaxel and the vinca alkaloids) and the antimalarials (quinine and artemisinin) are noteworthy (Ramwa et al 2009). Increasing awareness towards benefits of organic ingredients and concern related environmental sustainability, the cosmetics and personal care industry has witnessed a swift shift towards organic and natural products.

Deodorants and antiperspirants are designed to reduce the growth of microorganisms that cause odours. Though, there are risks that some of these substances might be harmful to human health. Since the 1950s, aluminium zirconium has been a component of aerosolized antiperspirants; nevertheless, the Food and Drug Administration (FDA) prohibited the substance in 1977 as a result of serious safety concerns. But in many parts of the world, antiperspirant treatments still contain identical aluminium compounds. An antibacterial ingredient used in deodorants called triclosan has lately been related to skin irritation and has been known to cause allergies in certain persons. Although extensive testing is being done, there are still issues with the topical application of chemical-based deodorants or antiperspirants. A potential replacement for the current formulations is to look at Natural alternatives to get around these issues (Wright et al., 2020).

The majority of Indians suffer from acne vulgaris, a chronic skin illness. This infection is caused by a combination of internal and external causes, including inflammation, hormonal imbalance, and external bacterial infection. The two main bacteria present on skin that cause acne are *Cutibacterium acnes* and *Staphylococcus epidermidis*. There are numerous topical and systemic treatments for acne. For the treatment of acne, there are many topical and oral medications on the market, including Clindamycin, Salicylic Acid, Isotretinoin, Erythromycin, Triclosan, Tetracycline, Minocycline, and Metronidazole. However, using these medications excessively over an extended period of time can cause bacteria to become more resistant to them. These medications have toxicity restrictions as well as adverse effects include skin dryness, headaches, nausea, etc. The introduction of natural, effective, and safe anti-acne medications is highly required to get around these restrictions (Vora et al., 2018).

We intend to use bio-actives from *Mammea suriga* for antibacterial activity against malodour/acne causing micro-organisms.

NATIONAL STATUS

Mammea suriga (Mammea longifolia) is Endemic to the Western Ghats. Endemic species *are* those that are restricted to a geographical area and do not occur naturally in any other part of the world. It grows wild in western India's evergreen woods, from Khandala to Malabar and Coimbatore, and is farmed in Assam, West Bengal, Orissa, and Uttar Pradesh. It is also found in Ceylon and the West Indies. As per CAMP (2001), IUCN RED List (2007) and ADMA RED list, *Mammea suriga* Kosterm is reported as a threatened tree species endemic to the Western Ghats (S. L. Shastri et al., 2020)

Mammea suriga Kosterm is a member of the Cluciaceae family. It's also known as Indian rose chestnut or Ceylon ironwood, and it's also known as Punnag (Sanskrit), Surangi (Hindi), and Suragi (Kannada) (Wikipedia contributors, 2022a). It's a medium-sized tree very popular for its fragrant flowers. It has Simple, opposite, densely coriaceous, dark green leaves with a rounded base and pronounced midrib, veins are few, inconspicuous, and very narrow. Tree species that are androdioecious have male flowers on one plant and bisexual flowers on the other. During the hot season, the cauliflorus growth appears from overall surface of stem which develop into attractive spherical buds that open into four-petaled white flowers (*Mammea Suriga (Buch.-Ham. Ex Roxb.) Kosterm. | Species*, n.d.)

The tree's fresh blossoms are used for both temple worship and personal beautification. Dry flowers are utilized in the perfumery industry because they keep their smell for a long period even after drying. (Kubal et al., 2013). The method for extraction of essential oils has been standardised by *Jadhav et al* in 2016. The major components of extracted oil were analysed by head space and GCMS analysis were Trans-Alpha-Bergamotene (32.32%), Kaurene (10.60%) and Squalene (07.74%). Recently (Pise & Thorat, 2022) employed supercritical fluid method for essential oil extraction of dried mammea flowers.

In Ayurveda it is popular as Surapunaga which has similar properties to Punaga (*Callophyllum inophyllum*). Flower buds have applications in bleeding disorders such as menorrhagia, nasal bleeding, piles. It is also used as pittahara -useful in gastric disorders. Bark decoction is used in ulcerative colitis. It is also used in skin disorders. It is used as a substitute for Nagkesar, Mesua ferra (Hebbar, 2022). Dried Surangi flowers are reportedly giving an export market

worth USD 2.2 million to USD 4 million to almost 800 farmer families across 8–10 villages in the two months period of March and April (Pise & Thorat, 2022)

Different significances of the *Mammea suriga* tree have been explored, such as extraction of natural dyes (Verenkar and Krishnana, 2020), an alternative source of shikimic acid (Kshirsagar et al., 2020) and as an ethnomedicinal plant for skin cure and arthritis treatment (P. G. Bhat et al., 2014; Pise & Thorat, 2022).

M. Suriga Stem bark and flower bud extract in petroleum ether were shown to have considerable antibacterial action against *Pseudomonas aeruginosa, Salmonella typhi, Streptomyces pneumoniae*, and *Vibrio cholerae* (Shastri et al., 2016). *Mammea suriga* root bark extract in various solvents like Petroleum ether, chloroform, ethyl acetate and ethanol were tested in vitro for antibacterial activity against *Staphylococcus aureus*, *Bacillus subtilis*, and *Cryptococcus neoformans.* These extract's strong antibacterial action may be due to the presence of active alkaloids and flavonoids. (Poojary et al., 2015). Flavonoids, saponins, and alkaloids are reported to be present in the aqueous and ethanol extracts of *M. suriga* root bark. Analgesic properties occur in alkaloids and triterpenes, and anticancer properties in saponins, flavonoids, and quinones whereas Tannins, alkaloids, flavonoids, and triterpenes have been reported to have antibacterial and anti-inflammatory activities (Mosquera-Chaverra et al., 2022).

The present study concentrates on antibacterial activity of *Mammea suriga* extracts against malodour/acne causing microorganisms.

As *M. suriga* seeds are available in abundance there is a demand from local community to develop a method for commercial application of *M. Suriga* seeds.

One more aspect of our study is proximate analysis of seeds as there is no literature available and accordingly its commercial application. These seeds will be analysed for their moisturizing activity.

INTERNATIONAL STATUS

A species with high genetic variety provides valuable preliminary selection materials for crop development. In addition to molecular markers, morphological, agronomical, and physiological traits can be used to determine genetic diversity. Although numerous elements, including the environment, the stage of the plant's development, and polygenic inheritance, have an impact on morphological characteristics, it was generally agreed that morphological characterization comes before beginning DNA-based studies. Establishing an effective conservation strategy for a species requires evaluation of its diversity, distribution range, and habitat factors. For creation of initiatives to protect genetic resources, knowledge of the amount and distribution of genetic variation within and among populations of a particular species may be crucial (Selim et al., 2021). Loss of genetic variability is a significant issue in the conservation of biological variety, because it prevents a species from responding to natural selection and restricts its capacity for evolution (Gul et al., 2021).

There are several reports where the phenotypic variability in endemic plants have been studied at population level and many have corelated it with geographical edaphic or environmental factors.

A rare and endangered species called *Phlomis aurea Decne* lives in the southern Sinai Peninsula of Egypt at high heights. Shaltout et al., 2015 tried to evaluate the impact of various habitats such as basins, mountain slopes, gorges, and wadi beds by examining the population structure of *P. aurea* in terms of size distribution, height, diameter, density, frequency, and cover. They showed that the population of *Phlomis aurea* in the basin had the second-highest density (behind of gorges) along with the highest cover and size index. This may be explained by the fact that the basin was a catchment area and received additional moisture and nutrients from the nearby elevated areas compared to the other habitats. The slope's P. aurea population had the lowest density, along with the lowest cover and size indices; this might be because of the slopes' notable stony characteristics, which frequently result in lower moisture availability due to their poor capacity to hold moisture, as well as loss of water and organic matter due to run-off effect. According to Abd El-Wahab (2006), the most crucial variables affecting the availability of soil nutrients and regulating the covering and structure of vegetation were altitude, soil pH, EC, silt, clay, water holding capacity, and organic matter.

Studies by Moustafa and Abd El-Wahab (2013), Moustafa (1990), Moustafa et al. (2001), Ramadan (1988), Abd El-Wahab (1995), Zaghloul (1997), and Migahid et al. (1958) established that vegetation characteristics and plant cover were positively connected with altitude.

Dorystoechas hastata is a relict endemic species grown in Antalya, Turkey. The species is under risk of extinction due mainly to the uncontrolled mass collection for its pharmacological properties. Selim et al., 2021 studied the morphological diversity of *D. hastata* in its natural habitat and the effect of habitat characteristics on the species diversity. The results showed existence of substantial variation in *D. hastata* for morphological characteristic and natural distribution from sea level up to 2000 m. The findings of the soil research showed that there were notable variations in soil characteristics amongst populations. The majority of populations mostly thrive on clay soils with pH values between 7.5 and 8.0, which are somewhat alkaline. One of the main landscape elements that affects the microclimate and potential habitat for vegetation is slope aspect. It affects incident solar radiation, which in turn modifies soil moisture, air and surface temperature.

The present study aims at Morphological variation in *M. suriga* within and between different populations and corelate with environmental conditions

Chemical substances known as phytochemicals are found naturally in the plant kingdom. The phrase is typically used to describe substances such as carotenoids, flavonoids, and coumarins that may be of biological importance. The benzopyrone family includes the coumarins. Coumarins are used as industrial additives, cosmetics, and fragrances. The development of coumarin-based anticoagulant, antioxidant, antimicrobial (anti-viral, anti-fungal, and anti-parasitic), anticancer, anti-diabetic, analgesic, anti-neurodegenerative, and anti-inflammatory medicines has received a lot of attention. (Matos et al., 2015)

Plants of the family Cluciaceae and their genera Mammea, Mesua, and Calophyllum are good sources of coumarins (Rouger et al., 2018)These genera produce mammea coumarins, molecules with a very restricted structure in the plant kingdom, with a particular and unique nomenclature. They are uniquely characterized as being hydroxylated at C-5 and C-7; substituted at C-4 with phenyl, propyl, pentyl, methylpropyl, or 1-acetoxypropyl residues and at C-6 and C-8 with an acyl or prenyl residue (geranyl in the case of surangin A and B). Based on the substituent at C-4, there are so far five designated series: mammea A (4-fenil), B (4-propil), C (4-pentil), D [4-(1- metilpropil)], and E (1-acetoxipropil) (Crombie et al., 1987). These

types of coumarins are biologically active with antiprotozoal activity against Leishmania amazonensis (Brenzan et al., 2007, 2012), L. brasiliensis (Honda et al., 2010) and Trypanosoma cruzi (Reyes-Chilpa et al., 2008); cytotoxic activity (Reyes-Chilpa et al., 2004; Ito et al., 2006; Du et al., 2010), and others have shown good activity against dengue and chikungunya viruses (Gómez-Calderón et al., 2017).

Anticancerous activity:

Two new naturally occurring coumarins, isomesuol (**1**) and mammearin A (**2**), together with nine known *Mammea* coumarins **3 - 11** were isolated from the EtOAc extract of the leaves and twigs of *Mammea harmandii.* Coumarins **1**, **3** and **4** showed cytotoxicity against a panel of mammalian cancer cell lines (Reutrakul et al., 2003).

Mammea A/AA isolated from Methanolic stem bark extract of *Mammea africana* exerts specific inhibitory activity against Campylobacter jejuni, Streptococcus pneumoniae, and Campylobacter difficile. Mammea A/AA was also found to exhibit significant cytotoxicity against both cancer and normal cell lines (Canning et al., 2013).

Testosterone 5α-Reductase Inhibitory Activity of Mammea coumarins:

The male sex hormones, androgens, play a crucial role in the development, growth and function of the prostate, and other androgen-sensitive peripheral tissues. In the prostate gland, androgens are involved in benign prostatic hyperplasia and prostate cancer, as well as in skin disorders, such as acne, seborrhea, androgenic alopecia, and hirsutism. Among the androgens, testosterone is the most abundant in serum and secreted primarily by the testicles and ovaries. The enzyme steroid 5α-reductase catalyzes the conversion of testosterone to the most potent natural androgen, 5α-dihydrotestosterone (Yamana et al., 2010). Therefore, inhibition of testosterone 5α-reductase could be useful for the treatment of the above diseases. Geranylated Coumarins From Thai Medicinal Plant *Mammea siamensis* were found to inhibit Testosterone 5α-Reductase Activity (Morikawa et al 2020).

Recently (Pájaro-González et al., 2022) extensively worked on bio-guided fractionation of the ethanol extract of seeds of *Mammea americana L* for the antibacterial activity, against S. aureus strains that are sensitive and resistant to methicillin. The most effective fraction contained coumarines Mammea B/BA which presented MIC values in a range between 0.5 and 1.0 μg/ml.

After Preliminary screening current study suggests that *Mammea suriga* extract are having an excellent anti-malodour/anti-acne activity. Our further objective is bio-guided fractionation

of methanol extract of *M. suriga* and the most potent fraction will be subjected to HPTLC -MS analysis or FTIR analysis to find out the probable substance responsible for anti-acne / anti-malodour activity.

M. Suriga seeds will be subjected for proximate analysis. These seeds will be analysed for their moisturizing activity.

STUDY HYPOTHESIS

Plant species distributed along wide environmental gradients often show large phenotypic variation reflecting their habitat heterogeneity. The phenotypic plasticity determines short-term morphological and physiological responses to environmental fluctuations. Different environmental selection pressures may lead to long-term evolutionary changes in phenotypes adapting to local environments. Phenotypic variation due to genetic differentiation along environmental gradients often underlies formation of ecotypes or intraspecific taxa, which are at initial stages of speciation. Thus, study on geographic variation of phenotypes is important to understand mechanisms of evolution and intraspecific diversity. (Hirano et al., 2017)

Environmental factors like Rainfall levels, environmental temperature and availability of nutrients can bring about the changes in plant architecture, growth and reproduction leading genetic and phenotypic variation in plant populations. Documenting spatial variation in vegetative and reproductive phenotypes, will allow us to understand evolutionary trends in plant species. (Arteaga et al., 2015)
This study analyses the variation in flowering and vegetative traits of *Mammea suriga* across its population in Konkan region and the effects of abiotic variables on phenotypic variation.

Sweat glands form in hairy areas such as the armpits, genitals, and scalp, and release an oily fluid containing proteins, lipids, and hormones. This viscous fluid (sweat) is almost entirely odourless, contrary to popular assumption. The malodorous by products that cause body odour are only produced when members of the skin microflora digest these secretions. Armpits provide a moist, warm habitat for bacteria to grow, making them a microbial hotspot in humans. Members of the genera *Corynebacterium, Staphylococcus,* and *Cutibacterium* are some of the most frequent bacteria that cause body odour. The formation of volatile organic compounds (VOCs), such as volatile fatty acids and thioalcohols, is mostly responsible for the odour. (Khanna, 2021) (Callewaert et al., 2017) Deodorant masks this stench and, depending on the fragrance of deodorant, it can even make consumer smell better. Deodorants provide two types of odour prevention. The first is antibacterial capabilities, which lower the quantity of

germs responsible for odour production. The second is a scent that conceals any odours that may be present. (Ratini, 2021).

Despite their effective antibacterial properties, common antibacterial agents frequently used in deodorant formulations such as triclosan and aluminum salts, increase the risk of Alzheimer's disease, breast and prostate cancer, and contact dermatitis. Plant extracts inhibiting odour causing bacteria are therefore of interest. The ingredients used in natural antiperspirant formulations include Lavender, sage, Cinnamomum, patchouli, shea butter, virgin coconut oil, Arrow root, baking soda etc.

There are many medicinal plants traditionally known to counter the problem of body odor and excessive sweating within the South-East Asia region. Despite their potential, very limited studies on the activity and efficacy of these natural ingredients were available. Recently (HENMI et al., 2020) screened number of plant extracts to identify novel deodorizing natural products and they shortlisted 1,8-Cinole from cluster mallow leaves as an effective agent for eliminating various unpleasant odours. Sidek et al., 2021 studied the *in vitro* antibacterial activity of ten medicinal herbs that have conventionally been used to treat body odour against the skin microbiota that causes body odour. *Piper betle, Syzygium aromaticum*, and *Curcuma xanthorrhiza* ethanol extracts inhibited the growth of three skin microbiota strains*, S. epidermidis, C. tuberculostearicum*, and *C. jeikeium* that cause body odour, making them promising medicinal plants for the creation of natural deodorants and antiperspirants.

The comorbidity of chronic skin conditions and mental health disorders has long been known to psychodermatologist. Acne vulgaris is one such dermatological condition allied with depression, anxiety, and other psychological sequelae. Acne vulgaris is considered the most frequent skin disease of sebaceous glands with characteristic inflammatory lesions on the face. A growing group of evidence stated that the presence of the obligated anaerobic Gram-positive bacterium *Cutibacterium acnes* (*P. acnes*) in the follicular and sebum canals is responsible for the development of inflammatory acne. Antibacterial and antioxidant strategies for acne treatment through plant extracts are well known (Vora et al., 2018). Recently (Nakyai et al., 2021) reported Anti-Acne Vulgaris Potential of the Ethanolic Extract of *Mesua ferrea* L. Flowers. Piramal Healthcare Limited (Sharma et al., 2015) has US patent application for method for treatment of acne and anti-acne formulations comprising of

coumarin based compounds derived from at least one plant material selected form *Mammea longifolia*, *Mammea americana*, *Mammea africana* and *Mesua ferrea*.

The present study aims at determining antibacterial potential of the endemic species *Mammea suriga* against malodour producing or acne causing microorganisms.

STUDY RATIONALE

The genus Mammea belonging to family Cluciaceae contains roughly 70 species. It includes evergreen trees. Some of the species are *Mammea africana*, *Mammea americana*, *Mammea Narvosa, Mammea Harmandii, Mammea Siamensis*, and *Mammea suriga*. Tropical America and the West Indies are home to *Mammea americana* and *M. immansueta*. There are about 20 species, many of which are found in Madagascar and mainland Africa, including *M. africana* and *M. usambarensis*. India's western ghats are home to *Mammea suriga* (Wikipedia contributors, 2022b)

The present communication deals with morphological diversity of *M. suriga* with-in and between various populations found in different habitats.

Traditional practitioners residing in the vicinity of the villages of the Western Ghats are using the stem bark extract of *M. suriga* for bleeding piles, fevers, foul breath, haemorrhoids, headache, and dyspepsia. Flower buds are used in many skin diseases like ringworm, intertrigo, eczema, dandruff (Bhat et al., 2014).

As discussed earlier in other species the antibacterial activity is corelated in coumarins which are extracted in polar solvents like ethanol, methanol, ethyl acetate etc. Piramal Healthcare Limited has US patent application for method for treatment of acne and anti-acne formulations comprising at least one active ingredient selected from the group consisting of coumarin based compounds. These compounds are derived from at least one plant material selected form the group consisting of *Mammea longifolia*, *Mammea americana*, *Mammea africana* and *Mesua ferrea*. The formulation is in the form of powder, cream, ointment, lotion, gel, cake, Stick, Soap and shampoo. The investigation made by (Shastri et al., 2016)revealed, the petroleum ether extracts of stem bark and flower buds of *M. suriga* showed promising antibacterial activity against clinically pathogenic S. Typhi (7.00±0.33) when compared to standard drug (7.00±0.00). By in silico analysis, it seems that Cholestan-19-ol,5,6-epoxy-3-fluoro- (3.beta.,5.alpha.,6.alpha.) is promoting the remarkable antibacterial activity through the inhibition of GlcN-6P synthase. This investigation supported the medicinal claim of *Mammea suriga* as an antibacterial drug. The extracts of *M. suriga* showed the presence of an abundant quantity of flavonoids, glycosides, and terpenoids (Shastri et al., 2016). *Mammea*

suriga root bark extract in various solvents like Petroleum ether, chloroform, ethyl acetate, ethanol and water showed potent antimicrobial activity (Poojary et al., 2015) and they have related it with rich alkaloids and flavonoid content.

Present work concentrates on following aspects

A) **Need for biodiversity study:** The ecological setting and endemism of the Western Ghats make it unique in the world. Since the Western Ghats have a complicated topography and a complex monsoon system, they offer an extremely varied habitat, according to *Munoz*. The stable zones act as natural gene reserves, making their protection crucial. When the climate changes in the future, natural adaptation may occur in these locations. These locations may be more susceptible to climate change if there is more human influence. It is because of the variety of environmental conditions the Western Ghats has offered that so many indigenous species have endured there for millennia. On the other hand, as a species disappears, the habitat may become more fragile. (Warrier, 2016)

B) **Antimicrobial activity against malodour causing/ acne causing microorganisms:** In present work different plant parts like flower, buds, seeds and leaves of *Mammea suriga* will be used for extraction in both polar and non-polar solvents. Initially these extracts will be screened for antimicrobial activity against malodour causing micro-organisms (*Staphylococcus epidermidis* and *Corynebacterium xerosis*) and anti-acne activity against *Cutibacterium acne*.

C) **Relating the antimicrobial activity with chemical analysis**: The best extracts will be shortlisted and subjected initially to TLC/HPTLC analysis and finally to column chromatography and derived fractions will be screened for antibacterial activity against mal odour causing/acne causing organisms.

D) **Toxicity studies:** Cosmetic preparation using natural extracts requires scientific backup concerning toxicity evaluation and efficacy determination. With increasing awareness, it has become extremely important to access the cytotoxicity of such cosmetic actives before their utilization in cosmetic formulations. Some of the *Mammea* coumarins like Mammea A/AA was also found to exhibit significant cytotoxicity against both cancer and normal cell lines (Canning et al., 2013) there is a

need to evaluate the cytotoxicity of the extracts which are potent in inhibiting malodour causing/ acne causing bacteria.

E) **Exploring cosmetic applications of Mammea seeds:** The special reason for including seeds for activity against mal odour causing/acne causing organisms is: The Mammea plant is dioecious, male plants bear staminate flowers and are precious for fragrance. The female plants bear hermaphrodite flowers which ultimately result into thousands of seeds. Until now no efforts have been made for utilization of seeds for some commercial application. There is no report on proximate analysis of the seeds. And local farmers are looking at academicians and researchers with high expectations for exploring these seeds for commercial applications.

RESEARCH METHODOLOGY

1) **Biodiversity Study**
   - *M. suriga* population will be selected from 3 geographical locations (Sindhudurg, Ratnagiri and Karnataka) depending upon the altitude variations (Low altitude coastal region, slopes region and hill region).
   - Different morphological characters like Tree height, Tree stem girth, Leaf morphology, Floral structure, Pollen structure, Olfactory evaluation of flowers and essential oil as well as GC profile will be studied.
   - Extraction of Concrete fragrant molecule from dry and fresh flowers of *M. Suriga* obtained from Kokan and Karnataka region using different solvents
2) **Extraction of active components from flowers, buds, leaves and seeds of *Mammea suriga* using various solvents**
   - Extraction of active components from flowers, buds, leaves and seeds of *Mammea suriga* using solvents like 80% methanol, methanol, Dichloromethane (DCM) and Hexane
   - These extracts will be subjected for anti-microbial analysis
3) **Evaluation of antimicrobial activity of extracts against malodour causing micro-organisms (*Staphylococcus epidermidis* and *Corynebacterium xerosis*) and anti-acne activity against *Propionibacterium acne*.**
   - Flowers and buds of *M. Suriga* will be collected from 2 different geographical locations Kokan and Karnataka.
   - Preliminary antimicrobial activity will be checked by ZOI (Zone of inhibition) method and best solvent for extracting the antimicrobial actives will be screened depending upon results of ZOI.
   - Further extracts from selected solvent will be subjected to analysis of minimum inhibitory concentration (MIC) and minimum lethal concentration (MLC) against selected bacteria.
4) **Characterization of Active component responsible for antimicrobial activity**

- Active component responsible for antimicrobial activity will be separated from *M. suriga* extracts using column chromatography and further analysed by TLC, HPTLC or HPTLC-MS for structural evaluation

5) **Proximate analysis of seeds of *Mammea suriga***
    - Qualitative analysis of polysaccharides present in seeds of *M. suriga* will be done using appropriate methods.

6) **Evaluation of *in-vitro* cytotoxicity of extracts on HDF (Human Dermal Fibroblast) cell line.**
    - Toxicity of *Mammea suriga* extracts will be checked on HDF cell line to determine the cell viability.

7) **Evaluation of anti-proliferative activity of the extracts on cancer cell lines.**
    - *M. suriga* extracts will be analysed for anti-cancerous activity.

8) **Determine the Antioxidant capacity of *M. Suriga* extracts**
    - Whether *M. suriga* extracts are having antioxidant activity or not will be analysed by DPPH methods

9) **Incorporation of extracts in suitable cosmetic formulations**

STATISTICAL PLAN

Depending upon the results appropriate parametric or non-parametric statistical test will be applied.

LIMITATIONS OF STUDY

- Conducting clinical trials for deo formulations are very costly.

EXPECTED OUTCOME

- Study of biodiversity of *M. Suriga* including morphological characteristics like Tree height, Tree stem girth, Leaf morphology, Floral structure, Pollen structure, Olfactory evaluation
- Comparative Antimicrobial activity of flowers and buds obtained from various regions of Kokan will be analysed.
- Proximate analysis of *M. Suriga* seeds and its application.
- The safe non-toxic concentration of *M. Suriga* extract will be added to formulation. The natural formulation for anti-malodour activity will be available.

Bibliography

Arteaga, M. C., Bello-Bedoy, R., León-de La Luz, J. L., Delgadillo, J., & Dominguez, R. (2015). Phenotypic variation of flowering and vegetative morphological traits along the distribution for the endemic species Yucca capensis (Agavaceae). *Botanical Sciences*, *93*(4), 765–770. https://doi.org/10.17129/botsci.214

Bhat, P. G., Hegde, G., Hegde, G., & Mulgund, G. S. (2014). Ethnomedicinal plants to cure skin diseases—An account of the traditional knowledge in the coastal parts of Central Western Ghats, Karnataka, India. *Journal of Ethnopharmacology*, *151*(1), 493–502. https://doi.org/10.1016/j.jep.2013.10.062

Bhat, P., Hegde, G. R., Hegde, G., & Mulgund, G. S. (2014). Ethnomedicinal plants to cure skin diseases—An account of the traditional knowledge in the coastal parts of Central Western Ghats, Karnataka, India. *Journal of Ethnopharmacology*, *151*(1), 493–502. https://doi.org/10.1016/j.jep.2013.10.062

Callewaert, C., Lambert, J., & Van De Wiele, T. (2017). Towards a bacterial treatment for armpit malodour. *Experimental Dermatology*, *26*(5), 388–391. https://doi.org/10.1111/exd.13259

Canning, C., Sun, S., Ji, X., Gupta, S., & Zhou, K. (2013). Antibacterial and cytotoxic activity of isoprenylated coumarin mammea A/AA isolated from Mammea africana. *Journal of Ethnopharmacology*, *147*(1), 259–262. https://doi.org/10.1016/j.jep.2013.02.026

Crombie, L., Jones, R. C. F., & Palmer, C. J. (1987). Synthesis of the Mammea coumarins. Part 1. The coumarins of the mammea A, B, and C series. *Journal of the Chemical Society, Perkin Transactions 1*, 317. https://doi.org/10.1039/p19870000317

Gómez-Calderón, C., Mesa-Castro, C., Robledo, S., Gómez, S., Bolivar-Avila, S., Diaz-Castillo, F., & Martínez-Gutierrez, M. (2017). Antiviral effect of compounds derived from the seeds of Mammea americana and Tabernaemontana cymosa on Dengue and Chikungunya virus infections. *BMC Complementary and Alternative Medicine*, *17*(1). https://doi.org/10.1186/s12906-017-1562-1

Gul, J., Saeed, S., Ahmed, A., Laghari, S. U. K., Basit, A., Rehman, A., & Zahid, M. (2021). Genetic diversity and morphological variation of Pinus gerardiana along the environmental gradient from Zhob, Balochistan, Pakistan. *Nusantara Bioscience*, *13*(1). https://doi.org/10.13057/nusbiosci/n130116

Hebbar, J. V. (2022). Surapunnaga – Uses, Side Effects. *Easy Ayurveda*. https://www.easyayurveda.com/2015/04/23/surapunnaga-uses-side-effects/

HENMI, A., SUGINO, T., NAKAMURA, K., NOMURA, M., & OKUHARA, M. (2020). Screening of deodorizing active compounds from natural materials and deodorizing properties of cineole. *J. Japan Association on Odor Environment*, *51*(2). https://ci.nii.ac.jp/naid/40022198624

Hirano, M., Sakaguchi, S., & Takahashi, K. (2017). Phenotypic differentiation of the*Solidago virgaurea*complex along an elevational gradient: Insights from a common garden experiment and population genetics. *Ecology and Evolution*, *7*(17), 6949–6962. https://doi.org/10.1002/ece3.3252

Jadhav, N., Thergaonkar, R., & Deodhar, M. (2016). Extraction of Essential Oil from Flowers of Mammea suriga(Buch.-Ham. Ex Roxb.) KOSTERM. GC-MS Analysis and Incorporation in Cosmetic Product. *Human Journals IJPPR*, *6*(4), 531–540. https://ijppr.humanjournals.com/

Khanna, K. (2021, December 30). *Microbial Origins of Body Odor*. ASM.org. https://asm.org/Articles/2021/December/Microbial-Origins-of-Body-Odor

Kshirsagar, P. R., Pai, S. R., Vyavahare, G. D., Bapat, V. A., & Desai, N. S. (2020). Prospecting alternative source of shikimic acid from clusiaceae of Western Ghats, India and evaluating discrepancies with reference to seasonal variation, developmental stage and sex of Mammea suriga. *Industrial Crops and Products*, *149*, 112354. https://doi.org/10.1016/j.indcrop.2020.112354

Kubal, S., Shirke, V., Shirke, K., Wanage, S., Gunaga, R., & Rane, A. (2013). Triple-seeds in Mammea suriga (Buch.-Ham. Ex Roxb.), an Avenue Tree. *Research and Reviews: Journal of Botanical Sciences*, *2*(1), 1–2. https://www.rroij.com/open-access/tripleseeds-in-mammea-suriga-buchham-ex-roxb-an-avenue-tree-.php?aid=33807

Mammea suriga (Buch.-Ham. ex Roxb.) Kosterm. | Species. (n.d.). India Biodiversity Portal. https://indiabiodiversity.org/species/show/14963

Matos, M. J., Santana, L., Uriarte, E., Abreu, O. A., Molina, E., & Yordi, E. G. (2015). Coumarins — An Important Class of Phytochemicals. *Phytochemicals - Isolation, Characterisation and Role in Human Health*. https://doi.org/10.5772/59982

Morikawa, T., Luo, F., Manse, Y., Sugita, H., Saeki, S., Chaipech, S., Pongpiriyadacha, Y., Muraoka, O., & Ninomiya, K. (2020). Geranylated Coumarins From Thai Medicinal Plant Mammea siamensis With Testosterone 5α-Reductase Inhibitory Activity. *Frontiers in Chemistry*, *8*. https://doi.org/10.3389/fchem.2020.00199

Mosquera-Chaverra, L., Salas-Moreno, M., & Marrugo-Negrete, J. (2022). Ethnomedicinal Studies, Chemical Composition, and Antibacterial Activity of the Mammea americana L. Bark in the Municipality of Cértegui, Chocó, Colombia. *Advances in Pharmacological and Pharmaceutical Sciences*, *2022*, 1–15. https://doi.org/10.1155/2022/9950625

Myers, N., Mittermeier, R. A., Mittermeier, C. G., Da Fonseca, G. a. B., & Kent, J. (2000). Biodiversity hotspots for conservation priorities. *Nature*, *403*(6772), 853–858. https://doi.org/10.1038/35002501

Nakyai, W., Pabuprapap, W., Sroimee, W., Ajavakom, V., Yingyongnarongkul, B. E., & Suksamrarn, A. (2021). Anti-Acne Vulgaris Potential of the Ethanolic Extract of Mesua ferrea L. Flowers. *Cosmetics*, *8*(4), 107. https://doi.org/10.3390/cosmetics8040107

Pájaro-González, Y., Oliveros-Díaz, A. F., Cabrera-Barraza, J., Fernández-Daza, E., Reyes, N., Montes-Guevara, O. A., Caro-Fuentes, D., Franco-Ospina, L., Quiñones- Fletcher, W., Quave, C. L., & Díaz-Castillo, F. (2022). Mammea B/BA Isolated From the Seeds of Mammea americana L. (Calophyllaceae) is a Potent Inhibitor of Methicillin-Resistant Staphylococcus aureus. *Frontiers in Pharmacology*, *13*. https://doi.org/10.3389/fphar.2022.826404

Pise, V. H., & Thorat, B. N. (2022). Supercritical fluid extraction of dried Surangi flowers (Mammea suriga). *Industrial Crops and Products*, *186*, 115268. https://doi.org/10.1016/j.indcrop.2022.115268

Poojary, M. M., Vishnumurthy, K. A., & Vasudeva Adhikari, A. (2015). Extraction, characterization and biological studies of phytochemicals from Mammea suriga. *Journal of Pharmaceutical Analysis*, *5*(3), 182–189. https://doi.org/10.1016/j.jpha.2015.01.002

Ratini, M. (2021, June 2). *Difference Between Deodorant And Antiperspirant*. WebMD. https://www.webmd.com/skin-problems-and-treatments/difference-between-deodorant-and-antiperspirant#091e9c5e821b0aec-1-2

Reutrakul, V., Leewanich, P., Tuchinda, P., Pohmakotr, M., Jaipetch, T., Sophasan, S., & Santisuk, T. (2003). Cytotoxic Coumarins from*Mammea harmandii*. *Planta Medica*, *69*(11), 1048–1051. https://doi.org/10.1055/s-2003-45154

Rouger, C., Derbré, S., & Richomme, P. (2018). Mesua sp.: chemical aspects and pharmacological relevance of prenylated polyphenols. *Phytochemistry Reviews*, *18*(1), 317–342. https://doi.org/10.1007/s11101-018-9594-9

Selim, C. (2021, April 16). *Morphological Diversity of Dorystoechas hastata, a Relict Endemic Species, Across Habitat Variability*. http://www.pjoes.com/Morphological-Diversity-of-Dorystoechas-hastata-na-Relict-Endemic-Species-Across,128582,0,2.html

Shaltout, K. H., Ahmed, D. A., & Shabana, H. A. (2015). Population structure and dynamics of the endemic species Phlomis aurea Decne in different habitats in southern Sinai Peninsula, Egypt. *Global Ecology and Conservation*, *4*, 505–515. https://doi.org/10.1016/j.gecco.2015.10.002

Sharma, S., Suthar, A., Salkar, K., & Kedar, S. (2015). *METHOD OF TREATMENT FOR ACNE AND AN ANT-ACNE FORMULATION* (Patent No. US 2015 0011619A1). US patent.

Shastri, S., Krishna, V., Kumar, R., Kumar, S., Venkatesh, & Pradeepa, K. (2016). Phytochemical analysis, antibacterial property and molecular docking studies of Mammea suriga kosterm. *World Journal of Pharmaceutical Sciences*, *4*(9), 331–340. http://www.wjpsonline.org

Shastri, S. L., Venkatarangaiah, K., & Sheshagiri, A. (2020). SEED GERMINATION AND MICROPROPAGATION STUDIES OF MAMMEA SURIGA KOSTERM. *The Journal of Microbiology, Biotechnology and Food Sciences*, *10*(2), 211–216. https://doi.org/10.15414/jmbfs.2020.10.2.211-216

Sidek, N. a. M., Berg, B. V. D., Husain, K., & Said, M. M. (2021). Antimicrobial Potential of Ten Medicinal Plant Extracts Against Axillary Microbiota Causing Body Odor. *Pharmacophore*, *12*(6), 1–5. https://doi.org/10.51847/zp6vxap5vr

Verenkar, N., & Krishnana, S. (2020). Extraction of natural dye from Mammea suriga and its application on silk and cotton fabrics. *Indian Journal of Fibre & Textile Research*, *45*(4), 402–410. http://nopr.niscair.res.in/handle/123456789/55890

Vora, J., Srivastava, A., & Modi, H. (2018). Antibacterial and antioxidant strategies for acne treatment through plant extracts. *Informatics in Medicine Unlocked*, *13*, 128–132. https://doi.org/10.1016/j.imu.2017.10.005

Warrier, G. S. (2016, May 11). *Endemic trees in Western Ghats show climate resilience*. India Climate Dialogue. https://indiaclimatedialogue.net/2016/05/11/endemic-trees-western-ghats-show-climate-resilience/

Wikipedia contributors. (2022a, July 21). *Mammea suriga*. Wikipedia. https://en.wikipedia.org/wiki/Mammea_suriga

Wikipedia contributors. (2022b, September 4). *Mammea*. Wikipedia. Retrieved September 11, 2022, from https://en.wikipedia.org/wiki/Mammea

Wright, M. H., Wood, A., Greene, A. C., & Cock, I. E. (2020). Growth Inhibitory Activity of Acronychia acidula F. Muell. Fruit Extracts Towards Malodour-forming Bacteria. *Pharmacognosy Communications*, *10*(2), 95–101. https://doi.org/10.5530/pc.2020.2.18

Yamana, K., Labrie, F., & Luu-The, V. (2010). Human type 3 5α-reductase is expressed in peripheral tissues at higher levels than types 1 and 2 and its activity is potently inhibited by finasteride and dutasteride. *Hormone Molecular Biology and Clinical Investigation*, *2*(3). https://doi.org/10.1515/hmbci.2010.035